CHEMOSTERILIZATION OF THE BOLL WEEVIL

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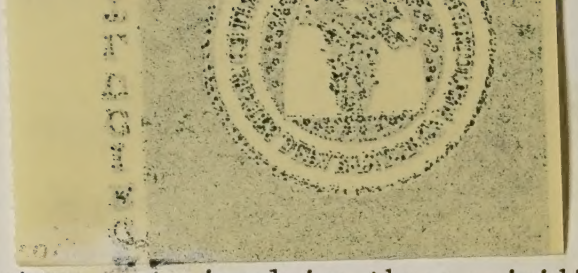

Various laboratory tests involving three aziridines (bisazir, thiotepa, and O-methyl bis(1-aziridinyl)phosphinothioate (AI3-50765)) and three benzoylureas (diflubenzuron, penfluron, and BAY SIR 8514 (AI3-29368)) were conducted to optimize the effectiveness of a fumigation-dip procedure developed specifically for producing sterile boll weevils suitable for field release studies. Data resulting from fumigation treatments with the aziridines, dip treatments with the benzoylureas, or treatments utilizing combinations of fumigation and dip techniques were tabulated and assessed.

KEYWORDS: Anthonomus grandis grandis Boheman, aziridines, AI3-50765, BAY SIR 8514, benzoylureas, bisazir, boll weevil, chemosterilants, chitin-synthesis inhibitors, compound application, diflubenzuron, fumigation-dip technique, penfluron, reproduction control, sterilants, sterilization techniques, thiotepa.

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INTRODUCTION

Chemical compounds that would decrease or eliminate the reproductive potential of the boll weevil, Anthonomus grandis grandis Boheman, could control this pest more effectively than insecticides (27, 28).2/ Since 1962 over 4000 chemicals were tested as candidate chemosterilants in this insect with over 100 of them showing distinct signs of sterilizing activity. Although the structures and properties of the active compounds are highly variable, several major chemical classes can be recognized.

Aziridines.--This largest group of insect chemosterilants (3) comprises over one-half of all the compounds active in the boll weevil. Apholate (2,2,=4,4,6,6-hexakis(1-aziridinyl)-2,2,4,4,6,6-hexahydro-1,3,5,2,4,6-triazatriphos=phorine) was one of the first effective male boll weevil sterilants (17, 23, 29, 32) but these sterilized weevils were short-lived and non-competitive. Many other active aziridines were reported (13, 16, 18, 21, 24, 26, 37, 40) but only bisazir (P,P-bis(1-aziridinyl)-N-methylphosphinothioic amide) was investigated in detail (5, 6, 7, 19, 31).

Alkanesulfonates.--Other than aziridines, the only other group of biological alkylating agents (4) that appeared promising for sterilizing boll weevils was alkanesulfonates (14, 18, 21, 32). Busulfan (1,4-butanediol dimethanesul=fonate), the most effective representative (11, 22, 25, 26, 34, 37),was the first chemosterilant used successfully in a large-scale field trial (30).

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2/ Underlined figures in parentheses refer to Literature Cited at the end of this report.

Nonalkylating phosphorus amides.--Hempa (hexamethylphosphoric triamide) was one of the first active sterilants in this category (11, 14, 17, 20, 21, 41) but only the initial results were promising. Similar to other phosphorus amides (13, 16, 18, 19, 37, 42),the sterility induced in the male weevils was not permanent.

Miscellaneous compounds.--Moderate to high activity was detected in a large variety of other compounds (1, 2, 9, 10, 12, 13, 22, 35, 38, 39), but since most of these compounds were effective primarily in female insects, their apparent practical potential was low. However, when the insecticidal properties of certain substituted N-(benzoyl)-N'-phenylureas were investigated in the boll weevil, it was discovered that the ovicidal effects of these materials were so powerful that they produced sterility in treated females. In contrast with other female boll weevil sterilants, the ureas had low mammalian toxicity and could be considered for direct field application. Diflubenzuron (N-[[(4-chlorophenyl)amino]carbonyl]-2,6-difluorobenzamide) was the first effective compound in this category (15, 33, 34), but its analog, penfluron (2,6-difluoro-N-[[[4-(trifluoromethyl)phenyl]amino]carbonyl]benzamide), was even more active and its sterilizing effect of longer duration (7, 36).

The discovery and availability of such a large number of sterilants with extremely variable physical and chemical properties led to a gradual development of methodologies for administering the compounds to the boll weevil. Feeding, the first technique demonstrated as being practical in large-scale releases (30), was time consuming and expensive, and consequently more rapid and logistically convenient methods were sought. Dipping insects in a solution of a sterilant has often been considered preferable to feeding, but little success was achieved before the development of a vacuum-dipping technique (31). Fumigation was another attractive alternative (5), though the necessity to utilize only compounds with significant vapor pressure at moderate temperatures severely reduced the choice of available sterilants. Vacuum fumigation (14) was an ingenious variation of the fumigation method, but it was too cumbersome for practical exploitation on a large scale. Eventually, the simplicity of fumigation and of dipping made possible the combining of the male-sterilizing properties of bisazir with the female-sterilizing properties of penfluron and the fumigation-dipping procedure was developed (7). Hundreds of tests exploring various aspects of boll weevil chemosterilization had to be designed and performed to evaluate this combined procedure. However, Borkovec, Woods, and Terry (7) were primarily concerned with the broad development of the methodology and did not include specific results. Here we report outlines and results of previously unpublished studies of the effects of penfluron, bisazir, and similar compounds on male and female boll weevils.

The first group of studies involves fumigation and the selection of fumigants most suitable for the purpose. The second group involves dipping experiments with penfluron. Finally, the third group includes studies of the combined fumigation-dipping technique and its evaluation.

MATERIALS AND METHODS

The boll weevils, produced at the Gast Rearing Laboratory, Mississippi State, Miss., were shipped weekly to our laboratories as mature larvae or pupae on diet plates. Emerging adult weevils were removed from cells in the plate and were segregated according to sex. Before and after treatment, weevils were housed in pint ice-cream cartons and were offered diet plugs at the rate of five weevils per plug. The plugs, which also served as oviposition media, were collected twice weekly and the deposited eggs extracted for evaluation. Collected eggs were incubated on moist filter paper to determine hatch or on diet plates to determine adult emergence. Experiments were normally carried out with 25 weevils per test. Details of test procedures were reported by Borkovec and McHaffey (5).

Fumigation Chamber

The fumigation chamber consisted of an electrically heated, cylindrical (20 x 20 cm), stainless steel desiccator (GCA/Precision Scientific Co., Cat. No. 68351). The air in the chamber was circulated by a rectangular blade constructed of two equal (9 x 11 cm) pieces of 8-mesh wire cloth, one of which was soldered to a drive shaft (0.64 cm diameter). Glass-fiber cloth (9 x 9 cm) was sandwiched between the wire cloth and the assembly was held together with two rubber bands. The drive shaft extending through a central hole in the cover was rotated by a variable speed stirrer motor operated at 600 rpm. Chamber temperature was controlled to ± 1° C by regulating the voltage applied to the heating circuit with a variable autotransformer which was periodically adjusted to correct for temperature drift.

The performance of this chamber was considerably improved over that of the apparatus previously described by Borkovec and McHaffey (5). For example, saturation of the chamber with chemosterilant vapor occurred within 10 min and recovered rapidly after chamber opening. Up to 3,000 weevils could be treated in the chamber without significantly changing the vapor concentration.

Fumigation Procedure

The boll weevils were confined, during fumigation, in stainless steel pill boxes (7.5 x 2.5 cm) with screen tops and bottoms to allow maximum air circulation and exposure of the weevils to the fumigant. The cages were placed horizontally in the chamber on top of a wire-cloth support, which was located 7 cm above the bottom and just below the stirring blade. The blade was assembled and the glass cloth charged by wetting it with a solution of 300 mg of the sterilant in 2 ml of methylene chloride. The blade was allowed to air dry for at least 30 minutes. The chamber, equipped with an air circulator having a solid blade, was loaded with caged weevils and the temperature adjusted. When the temperature remained constant, the sterilant-charged blade was installed and the fumigation procedure started. Tests were scheduled to open the chamber no oftener than every 30 minutes to remove weevils. Following fumigation, the weevils were placed in ice-cream cartons for mating or held for further treatment. Fumigants tested were thiotepa (tris(1-aziridinyl)=phosphine sulfide), bisazir, and AI3-50765 (O-methyl bis(1-aziridinyl)phosphi=nothioate).

Dipping Procedure

Groups of 25 boll weevils were placed in a cylindrically shaped glass cage (5 x 2.2 cm OD) fitted with a brass retainer screen cemented across the bottom. The loaded cage was dipped for 5 seconds into an acetone solution of the sterilant. Care was taken that all of the weevils were totally submerged. Weevils were removed from the cage, air dried on absorbent paper, and placed in ice-cream cartons. Compounds tested by dipping were diflubenzuron, penfluron, and BAY SIR 8514 (AI3-29368, 2-chloro-N-[[[4-(trifluoromethoxy)phenyl]=amino]carbonyl]benzamide).

Analysis of Residues

All analytical determinations of aziridine type compounds containing phosphorus were performed as follows: Five treated weevils were homogenized in a glass homogenizer with 3 successive 2-ml portions of acetone and the homogenates were filtered through a sintered-glass funnel of medium porosity. The combined filtrate was concentrated in a rotary evaporator and then quantitatively transferred to a 5-ml volumetric flask. The original compound and its metabolites, if any, were analyzed with a Hewlett-Packard 5710 Gas Chromatograph equipped with a flame photometric detector and a Hewlett-Packard Integrator by the method of Terry, McHaffey, and Borkovec (43). Two samples were processed and analyzed for each test.

RESULTS

Fumigation

Table 1 compares the effects of thiotepa and AI3-50765 with those of bisazir. In all tests, groups of 25 male weevils were fumigated and then crossmated with virgin females. At 90-minute exposure, the activity of the three compounds was comparable and all could be considered candidates for the combined fumigation-dipping procedure. Fecundity of females mated to the treated males did not differ significantly from that of the controls (not shown in table 1).

Dipping

Three aspects of the dipping procedure were investigated. The first series of tests concerned the choice of a most suitable compound selected from a group of substituted benzoylureas. Table 2 shows results obtained with diflubenzuron, BAY SIR 8514, and penfluron. Clearly, penfluron was the most effective sterilant when used as an acetone dip and treated females deposited fewer eggs than did the controls. A reduction in fecundity by the other compounds was also noticeable at increasing concentrations.

The second series of tests concerned the effect of penfluron on males that were mated in succession to four groups of virgin females. Table 3 shows that full sterility was induced in the first mating and that three further matings induced significant sterility. Fecundity was lowered in all tests but not as strongly as when the females were treated directly (table 2). Longevity of the males was not affected by the treatment.

Table 1.--Average mortality and sterility of male boll weevils fumigated (30° C) with thiotepa, AI3-50765, or bisazir for different exposure times 1/

Exposure time (min)	Percentage of mortality 2/			Percentage of hatch			Percentage of adult emergence		
	Thio-tepa	AI3-50765	Bisazir	Thio-tepa	AI3-50765	Bisazir	Thio-tepa	AI3-50765	Bisazir
30	18	26	--	24	21	--	13	18	--
45	48	43	--	16	8.6	--	10	3.7	--
60	44	48	84	14	.8	.7	10	.6	1.9
80	--	--	99	--	--	.1	--	--	0
90	52	65	56	.9	.03	.3	.3	0	0
100	--	--	100	--	--	0	--	--	0
120	--	84	90	--	0	.05	--	0	0
150	--	68	80	--	0	1.1	--	0	0

1/ Results from 1 to 4 replicates. Controls: Average mortality, 11 percent; average hatch, 59 percent; average adult emergence, 44 percent.

2/ Cumulative mortality 9 days posttreatment.

Table 2.--Average mortality, fecundity, and fertility of female boll weevils dipped (5 sec) in acetone solutions of diflubenzuron (DIF), BAY SIR 8514 (BAY), or penfluron (PEN) at various concentrations 1/

Concentration (ppm)	Percentage of mortality 2/			Number of eggs deposited			Percentage of hatch			Percentage of adult emergence		
	DIF	BAY	PEN	DIF	BAY	PEN	DIF	BAY	PEN	DIF	BAY	PEN
5,000	12	20	16	226	185	180	0	0	0	0	0	0
1,000	6	12	12	251	201	177	.7	0	0	0	0	0
200	10	8	12	324	272	170	2.6	7.7	0	0	0	0
40	8	24	11	336	384	183	15	69	.7	4.9	25	0
8	--	--	6	---	---	146	--	--	4.6	--	--	3.9
1.6	--	--	4	---	---	197	--	--	15	--	--	4.5

1/ Results of 2 to 3 replicates. Controls: Average mortality, 9 percent; average number eggs deposited, 305; average hatch,64 percent; average adult emergence, 27 percent.

2/ Cumulative mortality 15 days posttreatment.

Table 3.--Fecundity and fertility of four groups of virgin female boll weevils mated sequentially with males dipped (5 sec) in 1.2 percent penfluron in acetone 1/

Mating order 2/	Number of eggs deposited 3/	Percentage of hatch
1st	515	0
2d	507	15
3d	470	27
4th	517	6
Control	749	67

1/ Averages of two replicates. Mortality (43 days posttreatment): Treated males, 20 percent; control males, 48 percent.

2/ First mating occurred 1 hour posttreatment. Interval between successive matings, 7 days.

3/ From 25 females.

In the third series of tests, male weevils of different ages were treated. Table 4 shows that males of age 0-24 hours, 24-48 hours, or 48-72 hours responded to the treatment equally.

Fumigation and Dipping

An important question in the combined treatment procedure was the sequence of the two operations. Table 5 shows that when male weevils were first dipped in penfluron and then fumigated with bisazir, the uptake of the fumigant was sharply reduced compared with the reversed procedure. This difference was apparently responsible for the higher sterilizing efficiency of the fumigation-dipping sequence.

Because bisazir decomposes rapidly after the weevils are treated, analytical studies in the two operations had to be conducted at comparable conditions. As table 6 shows, uptakes of the fumigant, measured immediately after the fumigation-dipping or the dipping-fumigation procedures were concluded, are grossly different because in the former, the fumigant remained on and in the insect throughout the 3-hour rest period between fumigation and dipping. Consequently, the analyses for bisazir in the dipping-fumigation

Table 4.--Effect of age on mortality and sterilizing ability of male boll weevils dipped (5 sec) in 1.2 percent solution of penfluron and mated sequentially to virgin females 1/

Age at treatment (hours)	Percentage of mortality 43 days posttreatment	Mating sequence 2/							
		First		Second		Third		Fourth	
		Number of eggs	Percentage of hatch	Number of eggs	Percentage of hatch	Number of eggs	Percentage of hatch	Number of eggs	Percentage of hatch
0-24	28	586	0.3	345	22	687	39	645	17
24-48	28	476	0	369	8.1	486	33	684	6.7
48-72	24	522	.4	610	24	451	1.1	678	11
Control	60	661	59	---	--	---	--	---	--

1/ 25 males and females used in each test.

2/ First mating occurred 1-hour posttreatment; interval between successive matings, 7 days.

Table 5.--Effects of treatment sequence of fumigation and dipping on the mortality, sterility, and fumigant uptake of male boll weevils 1/

Sequence	Percentage of mortality 9 days posttreatment	Number of eggs deposited 2/	Percentage hatch	Percentage of adult emergence	Residue of fumigant 3/ (µg/♂)
Dip-Fumigation	34	460	0.8	0.9	0.30
Fumigation-Dip	44	499	.2	0	.57
Control	11	1093	54	49	--

1/ Results from 8 to 9 replicates. Fumigated 90 minutes at 30° C with bisazir. Dipped 5 seconds in 1.2 percent penfluron in acetone.

2/ From 25 females.

3/ Residue determined 3 hours after fumigation.

procedure (table 5) were conducted 3 hours after the procedure was terminated. When the analysis was performed 3 hours after simple fumigation, the residues of bisazir were almost identical to those obtained immediately after the fumigation-dipping procedure. Since the rest period in the latter procedure was 3 hours, the analyses were in each case conducted 3 hours after fumigation. Obviously, because the acetone-penfluron dip did not remove a significant amount of bisazir from the fumigated weevil, we conclude that the fumigated insects do not contain significant quantities of bisazir on their surface 3 hours after the fumigation-dip.

Table 6.--Uptake of bisazir by male boll weevils measured immediately after fumigation, dipping-fumigation, or fumigation-dipping, and 3 hours after fumigation 1/

Procedure	Interval (hour) between treatment termination and analysis	Residue of bisazir (µg/♂)
Fumigation	0	1.0
Do	3	.58
Dipping-fumigation	0	.66
Do	3	.30
Fumigation-dipping	0	.57

1/ Average of two replicates. Weevils fumigated 90 minutes at 30° C, dipped 5 seconds in 1.2 percent penfluron in acetone.

The effects of the rest period between fumigation and dipping were determined in a series of tests with periods ranging from 0.5 to 24 hours. As table 7 shows, no differences were observed except that mortality in tests with the longest period appeared excessive. In the course of this and other investigations, the necessity for establishing accurate mortality curves became apparent. Figure 1 summarizes the results of a large number of tests in which male boll weevils were treated by a standard procedure and their mortality was recorded. Effects of the length of fumigation on mortality were predictable and expected; however, the general shape of the mortality curves was of greater interest. The steep slope that begins in all treatments at 7 to 9 days posttreatment explained the apparent anomaly of the high mortality figure in table 7. As figure 1 shows, the mortality between days 9 and 10 posttreatment increases substantially and since the weevils in the 24-hour group were fumigated 1 day earlier than those in the 0.5 hour group, their mortality should correspond to the 10-day-posttreatment insects rather than to the 9-day-posttreatment ones. It should be noted that mortality was one of the most variable features of the test insects and that only extensive repli-

cations spanning months or years of weevil supply can provide satisfactory results.

Table 7.--Effects of various intervals between fumigation and dipping on the mortality and fertility of male boll weevils 1/

Interval (hour)	Percentage of mortality 9 days posttreatment	Number of eggs deposited 2/	Percentage of hatch	Percentage of adult emergence
24	64	366	0.1	0
3	20	590	.1	0
.5	24	413	0	0
Control	12	1,089	56	50

1/ Results from 3 to 6 replicates. Weevils fumigated 90 minutes at 30° C with bisazir and dipped 5 seconds in 1.2 percent penfluron in acetone.

2/ From 25 female weevils.

Mating competitiveness of male weevils treated by fumigation followed by dipping was determined in tests with different ratios of treated and untreated males combined with untreated females. Table 8 shows results of one such study with treated:untreated male ratios 1:2, 2:1, and 10:1. The calculated values in the last column were based on the assumption that treated and untreated males competed equally for the available females. However, as data in the observed column show, the treated males appeared to be much more competitive than the untreated ones.

DISCUSSION

In a long series of fumigation studies with male boll weevils, Borkovec and McHaffey (5) selected 13 volatile aziridinyl compounds that showed promise as sterilants. We investigated further the 3 most effective compounds in this group and as table 1 shows, all were sufficiently active to warrant inclusion in the fumigation-dipping procedure. Nevertheless, the advantages of bisazir pointed out by Borkovec and coworkers (7) and the inordinate effort that would have been required for full investigation of all three compounds as the combined procedure candidates led to the selection of bisazir for further detailed evaluation. The choice of the dipping agent (table 2) was much simpler because penfluron was clearly the most effective candidate. Its effect on the male, i.e., the ability of treated males to transfer penfluron to females in at least four subsequent matings (table 3), was undoubtedly responsible for the apparent hypercompetitiveness exhibited by treated males in the competitive mating test (table 8). Borkovec and coworkers (7) reported similar tests and results.

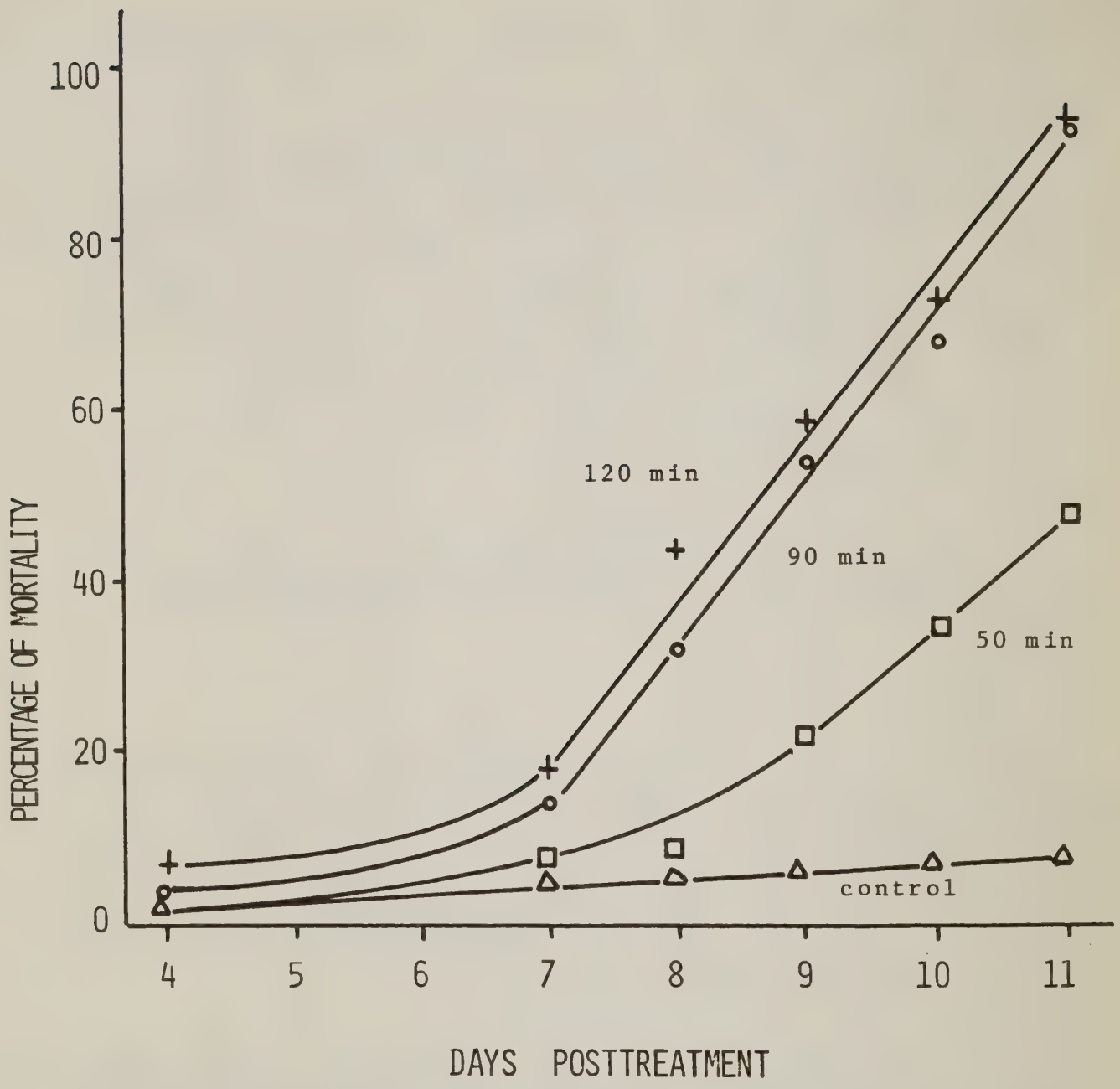

Figure 1.--Cumulative mortality of male boll weevils fumigated with bisazir for 50, 90, and 120 min and dipped in acetone solution of penfluron 30 min after fumigation (60-20 replicates).

Table 8.--Mating competitiveness of male boll weevils fumigated with bisazir and dipped in penfluron. Treated (T) and untreated (U) males in groups of 22-30 were combined with groups of 10 virgin females at indicated ratios 1/

T♂ :	U♂ :	U♀	Number of eggs deposited 2/	Percentage of hatch	
				Observed	Expected
10	20	10	288	63	46.6
20	10	10	473	9.9	23.3
20	2	10	322	1.2	7.0
--	30	10	376	70	---

1/ Males fumigated 90 minutes at 30° C and dipped 5 seconds in 1.35 percent penfluron in acetone.

2/ From 10 female weevils.

The effects of penfluron in dipping or fumigation-dipping treatments on fecundity of females is yet to be clarified. Whether the female is treated directly (table 2) or whether it only mates with a treated male (tables 3, 5, and 7), fecundity decreases and the decrease is even more noticeable in combined treatments (tables 5 and 7). Since penfluron and other chitin-synthesis inhibitors could be considered for direct field application as boll weevil pesticides, the reduction in fecundity may exert a beneficial population suppressing effect in addition to their ovicidal and other insecticidal activities. On the other hand, in the sterile male release procedure, the oviposition suppressing effects of penfluron as well as the fumigation treatment may indicate some abnormality in the mating process that would need further study.

Although an acceptable fumigation-dipping procedure applicable for the sterilization of mass-reared boll weevils has been developed (7), further improvements in this technique are possible should it be adopted for use in a large-scale eradication program (8). Procedures similar to those outlined in the present paper could then be utilized to achieve such improvements.

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